Pancreatic endocrine tumors: improved TNM staging and histopathological grading permit a clinically efficient prognostic stratification of patients

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Pancreatic endocrine tumors are rare diseases and devising a clinically effective prognostic stratification of patients is a major clinical challenge. This study aimed at assessing whether the tumor-node-metastasis (TNM)-based staging and proliferative activity-based grading recently proposed by the European NeuroEndocrine Tumors Society (ENETS) have clinical value. TNM was applied to 274 patients with histologically diagnosed pancreatic endocrine tumors operated from 1991 to 2005, with last follow-up at December 2007. According to World Health Organization (WHO) classification, 246 were well-differentiated neoplasms (51 benign, 56 uncertain behavior, 139 carcinomas) and 28 poorly differentiated carcinomas. Grading was based on Ki67 immunohistochemistry. Survival analysis not only ascertained the prognostic value of the TNM system but also highlighted that in the absence of nodal and distant metastasis, infiltration and tumor dimensions over 4 cm had prognostic significance. T parameters were then appropriately modified to reflect this weakness. The 5-year survival for modified TNM stages I, II, III and IV were 100, 93, 65 and 35%, respectively. Multivariate analysis identified TNM stages as independent predictors of death, in which stages II, III and IV showed a risk of death of 7, 29 and 58 times higher than stage I tumors ($P < 0.0001$). Ki67-based grading resulted an independent predictor of survival with cut-offs at 5 and 20%. In conclusion, WHO classification assigns clinically significant diagnostic categories to pancreatic endocrine tumors that need prognostic stratification by applying a staging system. The ENETS–TNM provides the best option, but it requires some modifications to be fully functional. The modified TNM described in this study ameliorates the clinical applicability and prediction of outcome of the ENETS–TNM; it (i) assigns a risk of death proportional to the stage at the time of diagnosis, and (ii) allows a clinically based staging of patients, as the T parameters as modified permit their clinical-radiological recognition. Ki67-based grading discerns prognosis of patients with same stage diseases.

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Pancreatic endocrine tumors are rare and clinically taxing diseases, as their natural history is largely

unknown.^{1–4} Affected patients seek medical assistance due to symptoms resulting from either hormonal hypersecretion or mass. Thus, pancreatic endocrine tumors are clinically defined as functioning or nonfunctioning depending on the presence of a syndrome related to inappropriate hormone secretion. Such distinction, however, does not provide prognostic information. Pancreatic endocrine tumors are histologically divided into well-

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differentiated or poorly differentiated neoplasms.^{1,5} The latter are invariably life-threatening diseases that rapidly metastasize, whereas the former include 90% of cases whose clinical behavior varies from indolent to highly malignant but cannot be predicted by their histology.^{1,5}

The clinical challenge resides in devising an efficient categorization of pancreatic endocrine tumors, which permits the planning of tailored surgical or medical therapies and follow-up programs. The preferred therapy for pancreatic endocrine tumors is curative surgical resection, only possible in 40% of cases. These patients demand follow-up, personalized on the basis of risk of relapse. In contrast, patients with unresectable disease are candidates for diverse medical treatments that range from use of somatostatin analogs, as most cases express somatostatin receptors, to variably aggressive chemotherapy regimens or peptide receptor radionuclide therapy.^{6–11} The open question is how we can best identify patients to treat with somatostatin analogs and those who will benefit from more aggressive therapies as first-line treatment.

The World Health Organization (WHO) classification of pancreatic endocrine tumors identifies three categories: well-differentiated endocrine tumor with either benign or uncertain behavior, well-differentiated endocrine carcinoma and poorly differentiated endocrine carcinoma.^{1,12} Histopathology discriminates poorly differentiated from well-differentiated neoplasms, but cannot distinguish further categories within the latter, and the term carcinoma is assigned only when invasion of extrapancreatic structures or lymph node metastasis are documented through the analysis of a surgically resected tumor. In patients who cannot profit from surgery, the discrimination between well-differentiated endocrine tumor and well-differentiated endocrine carcinoma is based on the clinical and radiological documentation of locally aggressive or metastatic disease.^{13,14} As under the term well-differentiated endocrine carcinoma are grouped cancers with variable behavior, there is the need of additional tools to distinguish prognostic classes particularly within this WHO category.

In the attempt to permit a stratification of pancreatic endocrine tumors into clinically significant risk categories, a staging system based on tumor-node-metastasis (TNM) parameters and a grading system based on the assessment of the proliferative activity of neoplastic cells have been proposed by the European NeuroEndocrine Tumors Society (ENETS).¹⁵ The ENETS–TNM system classifies tumors limited to the pancreas as T1, T2 or T3 based primarily on dimensions (<2, 2–4, >4 cm). T3 also includes cases with invasion of the duodenum or bile duct, whereas T4 cases are those with invasion of adjacent organs or large vessels. The proposed staging based on this TNM is as follows: stage I, T1N0M0; stage IIa, T2N0M0; stage

IIb, T3N0M0; stage IIIa, T4N0M0; stage IIIb, anyTaN1M0; stage IV, anyTaN1M1. The ENETS-grading system is based on the assessment of the proliferative activity measured by either the mitotic count or Ki67 immunohistochemistry and assigns neoplasms to one of the three categories: G1 if proliferative index is $\leq 2\%$; G2 if > 2 to 20% ; G3 if $> 20\%$.¹⁵

The validation of these TNM and grading systems has been variably addressed in four recent studies that all evidenced clear-cut distinctions only between higher and lower stages while they were of little help for intermediate-stage diseases.^{16–19} There is clearly the need of a system permitting the prognostic stratification of all pancreatic endocrine tumor patients, including both those who may benefit from radical surgery and those with more advanced disease that cannot be resected.

Our study aimed at assessing whether the proposed ENETS–TNM-based staging system when specifically applied to a nonselected prospective series of 274 pancreatic endocrine tumors: (i) has clinical value; (ii) can be applied to both resected and nonresected patients; (iii) adds prognostic information to WHO categorization; (iv) can be further improved by tumor grading based on the assessment of proliferative activity.

Patients and methods

The Series

The study included 274 consecutive patients suffering from pancreatic endocrine tumors who were operated (197 resections, 77 palliative or explorative) between 1991 and 2005 at Verona University Hospital. All neoplasms were of pancreatic origin, and patients with tumors arising from the papilla of Vater, bile duct or duodenum were excluded. All tumors were sporadic, and patients with MEN1 or von Hippel–Lindau syndrome were excluded from the study. All data were prospectively collected at Verona or Roma Universities, according to where patients were first observed, on a common computerized database. Tumor staging was performed by conventional imaging procedures, including ultrasonography, computed tomography and/or magnetic resonance, and was completed in all cases with the anatomical assessment of local or metastatic spread during surgery. From 1998, all patients underwent Octreoscan®. Follow-up was updated every 6 months, or at shorter intervals if necessary, and stopped in December 2007. The cause of death was verified by review of death certificates, hospital records or contact with the attending physicians or family. The diagnosis of pancreatic endocrine tumor was based on conventional histology and immunohistochemistry (Chromogranin A, Synaptophysin, NSE) on surgical specimen or biopsy.

WHO Classification, TNM Staging and Grading of the Series

All cases were reviewed and classified according to the criteria of the WHO¹ and were assigned to an ENETS–TNM-based stage and grading score.¹⁵ The proliferative index was expressed as a percentage based on the count of Ki67-positive cells in 2000 tumor cells in areas of the highest immunostaining, using the MIB1 antibody (DBA, Milan, Italy).

Statistical Analysis

Distribution of continuous variables is reported as median and interquartile range (IQR) (25th–75th percentiles). Categorical variables are presented as numbers and percentages. The comparison between subgroups was carried out using Student's *t*-test, or Mann–Whitney *U*-test for continuous variables. Qualitative data were compared by χ^2 -test or Fischer exact test when necessary.

The main outcome considered was survival, defined as the time from surgery to disease-specific death, and was censored at the last follow-up date if no events had occurred. Seven patients who died of causes other than cancer were considered censored at last observation. Follow-up was updated until 31 December 2007, giving a potential minimum follow-up of 12 months to each survivor. Follow-up time, in survival analysis, was stopped at 120 months.

Survival probability was estimated according to the Kaplan–Meier method, whereas the log-rank test was used for comparison of survival in different subgroups. Univariate and multivariate analyses were performed using the Cox regression model to evaluate significant 10-year mortality predictors. Concordance (*c*) index,²⁰ defined as the proportion of all usable patient pairs in which predictions and outcomes are concordant, was calculated for different Cox's models. The *c* index estimates the probability of concordance between predicted and observed survival. A value of 0.5 indicates no predictive discrimination, and a value of 1.0 indicates a perfect separation of patients with different outcomes. *P*-values were considered significant when less or equal than 0.05.

Results

The clinical–pathological characteristics of our series are summarized in Table 1. Tumors were 216 nonfunctioning and 58 functioning. These latter comprised 45 insulinomas, 6 gastrinomas, 3 glucagonomas, 2 somatostatinomas and 2 VIPomas. All the noninsulinoma functioning tumors were well-differentiated endocrine carcinomas. Postoperative mortality was nil. The median follow-up for survivors was 55.5 months (IQR 32–88). At the end follow-up, 182 patients were alive, 85 (31%) had died of disease and 7 died of other causes. The

Table 1 Clinicopathological data in 274 patients with pancreatic neuroendocrine tumors

	Total, n = 274 (%)
<i>Gender</i>	
Male	123 (45)
Female	151 (55)
Age at diagnosis (years) ^a	56 (43; 65)
<i>Site of neoplasm</i>	
Right pancreas	115 (42)
Left pancreas	159 (58)
<i>Type of neoplasm</i>	
Nonfunctioning	216 (79)
Functioning noninsulinoma	19 (7)
Insulinoma	39 (14)
<i>Resection</i>	
Resection	197 (72)
Palliative or explorative surgery	77 (28)
<i>WHO classification</i>	
Well-differentiated endocrine tumor—benign	51 (19)
Well-differentiated endocrine tumor—uncertain behavior	56 (20)
Well-differentiated endocrine carcinoma	139 (51)
Poorly differentiated endocrine carcinoma	28 (10)
Ki 67 (%) (<i>n</i> = 237) ^a	2 (1; 6)
<i>Status (end of follow-up)</i>	
Alive	182 (66)
Dead of cause other than cancer	7 (3)
Dead of disease	85 (31)

^aMedian (interquartile range).

overall 1-, 5- and 10-year survival was 90, 69 and 46%, respectively. The disease-specific mortality was 10, 30 and 47%, respectively.

Univariate Analysis on Determinants of Mortality

The results are reported in Table 2. Tumor dimensions showed a significant inverse correlation with mortality. However, Kaplan–Meier analysis did not show any difference in survival for tumors within 4 cm (10-year, size ≤ 2 cm: 85% vs 2.1–4 cm: 74%; *P* = 0.253). Moreover, there was a relationship between tumor dimensions and presence of infiltration. Neoplasms ≤ 2 cm were associated with infiltration of peripancreatic structures in 8% of cases (7/91), tumors between > 2 and ≤ 4 cm in 32% (20/62) and neoplasms > 4 cm in 85% (103/121) (Spearman's correlation: 0.69; *P* < 0.00001).

The T, N and M factors were associated with prognosis. Kaplan–Meier analysis showed a significant difference between the survival rates of patients with T1/T2 vs T3 or T4 neoplasms, as well as between T3 vs T4 (*P* < 0.05). No significant difference was found between the survival rates of patients with T1 and T2 neoplasms (10-year, 91 vs 82%; *P* = 0.346). Both N1 and M1 were predictors of

Table 2 Univariate analysis on determinants of mortality (left section) and Kaplan–Meier survival analysis (right section) in 274 patients affected by pancreatic endocrine tumor

	Alive (182 patients) and dead for other causes (7 patients) (%)	Dead of disease (85 patients) (%)	Cumulative survival rate (%)			
			1-year	5-year	10-year	P-value
<i>Gender</i>						
Male	84 (68)	39 (32)	85	68	47	0.240
Female	105 (70)	46 (30)	95	71	59	
<i>Type of neoplasm</i>						
Insulinoma	37 (95)	2 (5)	100	94	94	0.001
Functioning noninsulinoma	13 (68)	6 (32)	89	70	56	
Nonfunctioning	139 (64)	77 (36)	88	65	45	
<i>Neoplasm size</i>						
≤ 2 cm	83 (91)	8 (9)	98	88	88	<0.0001
> 2 cm ≤ 4	51 (82)	11 (18)	95	85	74	
> 4 cm	55 (45)	66 (55)	81	48	17	
<i>Neoplasm site</i>						
Right pancreas	75 (65)	40 (35)	90	65	44	0.162
Left pancreas	114 (72)	45 (28)	89	72	61	
<i>Ki67 (%) (n = 237)</i>						
0–2	117 (90)	13 (10)	98	90	80	<0.0001
> 2 to 20	54 (64)	31 (36)	89	63	46	
> 20	4 (18)	18 (82)	68	12	—	
<i>T factor</i>						
T1	69 (96)	3 (4)	100	94	91	<0.0001
T2	49 (89)	6 (11)	98	91	82	
T3	27 (77)	8 (23)	94	77	64	
T4	44 (39)	68 (61)	79	42	19	
<i>N factor</i>						
N0–NX	154 (90)	17 (10)	99	94	77	<0.0001
N1	35 (34)	68 (66)	75	31	20	
<i>M factor</i>						
M0	155 (85)	27 (15)	96	88	72	<0.0001
M1	34 (37)	58 (63)	77	35	17	

poor outcome with a 10-year survival of 20 and 23%, respectively.

Ki67 index resulted a prognostic factor. However, there was no difference in survival for Ki67 levels within 5%. In fact, the 10-year survival of patients with Ki67 ≤ 2% was 80% vs Ki67 2.1–5% that was 95% ($P = 0.487$).

Multivariate Analysis on Predictors of Mortality

Cox regression model adjusted for gender, age, site, size, type of neoplasm (functioning or not), nodal and distant metastases, identified four significant predictors of cancer-related mortality: dimensions > 4 cm (HR = 2.4; 95% CI = 1.04–5.51; $P = 0.040$), nodal involvement (HR = 4.7; 95% CI = 2.00–11.40; $P < 0.0001$), distant metastasis (HR = 2.2; 95% CI = 1.12–4.5; $P = 0.023$) and Ki67 with cut-offs at 5 and 20% (Ki67 > 5 to 20%: HR = 2.6; 95%

CI = 1.34–5.12; $P = 0.005$; Ki67 > 20%: HR = 8.0; 95% CI = 3.57–18.28; $P < 0.0001$).

Survival According to the WHO Classification, Original ENETS–TNM Staging and Ki67 Grading

The results are summarized in Table 3. For WHO classification, univariate analysis showed no difference in cancer-related mortality between well-differentiated endocrine tumors of benign or uncertain behavior. Both well-differentiated endocrine carcinomas and poorly differentiated endocrine carcinomas showed a significantly higher mortality with a median survival of 5.9 years (95% CI 4; 7.8) and 1.3 years (95% CI 0.8; 1.8), respectively, whereas only one patient (2%) among well-differentiated endocrine tumors of uncertain behavior and none with a well-differentiated endocrine tumor-benign died. Cox regression revealed WHO

Table 3 Multivariate survival analysis on 274 patients with pancreatic endocrine tumors

	Number of cancer-related deaths (%)	Cumulative survival rate (Kaplan–Meier)			Adjusted relative risk (Cox model)	P-value
		1-year	5-year	10-year	HR (95% CI)	
WHO classification ^a						
Well-differentiated endocrine tumor—benign	0/51 (0)	100	100	100	—	
Well-differentiated endocrine tumor—uncertain	1/56 (2)	100	100	95	1	
Well-differentiated endocrine carcinoma	62/139 (45)	89	60	31	2.23 (5.01–293.7) ^c	0.014
Poorly differentiated endocrine carcinoma	22/28 (79)	64	14	14	5.92 (5.28–511.56) ^c	<0.0001
ENETS–TNM stage ^b						
Stage I	1/67 (2)	100	100	96	1	
Stage IIa	1/41 (2)	100	100	92	1.17 (0.07–18.76) ^d	0.911
Stage IIb	2/16 (12)	100	93	74	3.12 (0.19–50.73) ^d	0.423
Stage IIIa	12/25 (48)	92	70	13	17.53 (2.02–152.24) ^d	0.009
Stage IIIb	8/29 (28)	86	59	—	8.97 (1.04–77.7) ^d	0.046
Stage IV	61/96 (63)	77	35	18	35.51 (4.59–274.45) ^d	0.001
Grade (Ki 67) (n = 237)						
G1 (0–2%)	13/130 (10)	98	90	80	1	
G2 (> 2 to 20%)	31/85 (36)	89	63	46	0.79 (0.39–1.59) ^e	0.506
G3 (> 20%)	18/22 (82)	68	12	—	5.34 (2.38–11.98) ^e	<0.0001

^aWHO classification.¹^bENETS–TNM staging system.¹⁵^cAdjusted for gender, age, site of neoplasm, size of neoplasm, type of neoplasm (functioning or not).^dAdjusted for gender, age, site of neoplasm, type of neoplasm (functioning or not), Ki67% levels.^eAdjusted for gender, age, site of neoplasm, type of neoplasm (functioning or not), TNM stage.

classification as a significant 10-year mortality predictor. The absence of any events among patients with well-differentiated endocrine tumor-benign excluded this category from calculation within the model. Nevertheless, no difference in prognosis was observed between well-differentiated endocrine tumors of benign or uncertain behavior subgroups as shown in Figure 1.

For ENETS–TNM staging, univariate survival analysis indicated a poorer survival directly related to higher stages. Multivariate analysis showed a significant difference between stage I/II and stage III or IV, as well as between stages III and IV. There was no difference in death probability between stages I and IIa and/or IIb tumors (Table 3).

For Ki67 grading, multivariate analysis highlighted a significantly shorter survival only for G3 neoplasms, while it did not distinguish between G1 and G2 when cut-off levels of 2 and 20% for Ki67 are used (Tables 2 and 3). Cut-offs at 5 and 20% were the most efficient in discerning prognostic differences between each grading category (Figure 1). Namely, G2 and G3 neoplasms showed significantly shorter 5-year and 10-year survival at Kaplan–Meier analysis and a respective 2-fold and 10-fold higher risk of death.

Insulinomas had a better outcome with respect to the other pancreatic endocrine tumor types, as expected. However, neither the presence of insulinomas in the cohort nor their location influenced the outcome of the Cox multivariate analysis, which was not affected when the 39 insulinomas were excluded (data not shown). The results were also not affected by the location of T3 and T4 neoplasms (data not shown).

Modifications to the ENETS–TNM Staging

The application of the original ENETS–TNM staging highlighted that in the absence of lymph node and distant metastasis, neoplastic infiltration and tumor dimensions over 4 cm had an independent prognostic significance. Thus, three modifications were applied to T code in order to more clearly stratify patients: (a) T3 was only defined by tumor dimensions >4 cm, excluding the infiltration of duodenum or bile duct, the presence of which assigns the case to T4; (b) T4 was defined by the recognition of cancer invasion of any adjacent structure, including duodenum and bile duct; (c) duodenum and bile duct involvement is also considered when assessed

Figure 1 Cumulative survival probability of 274 patients with pancreatic endocrine tumors according to the WHO classification (upper panel), the modified TNM staging system (middle panel) and the Ki67 levels (lower panel) according to Tables 4 and 5 of this paper. Probability curves were generated using Cox regression model adjusted for gender, age, site, size, type of neoplasm (functioning or not), nodal and distant metastases, and Ki67% levels.

by clinical (jaundice or duodenal bleeding due to infiltration) or radiological (dilation of pancreatic or bile duct) means in addition to the histopathological ones of the original proposal. The first two modifications separate potentially malignant neoplasms limited to the pancreas (T1–T3 categories) from overtly malignant diseases infiltrating extrapancreatic structures (T4). The third modification permits the application of TNM also to patients who cannot undergo surgery, where only clinical and imaging data are available.

Modifications to the ENETS Ki67 Grading

A fourth modification regarded the cut-off for Ki67 grading, as univariate and multivariate analyses on

determinants of mortality as well as survival analysis using Cox's model pointed that cut-off values at 5 and 20% were the most efficient in discrimination of prognostic categories.

Survival According to Modified TNM Staging and Grading

The results of univariate and multivariate survival analyses applied to the modified TNM staging are summarized in Table 4.

Two models were used. Model 1 considered the modified TNM staging alone. Model 2 adds the information obtained by applying grading based on Ki67 at 5 and 20% cut-off to the first model. Concordance analysis showed how the multivariate model that uses TNM coding alone (Table 4, model 1) had a good level of concordance between predicted and observed survival ($c = 0.72$; 95% CI = 0.67–0.78; $P < 0.0001$). The addition of Ki67 grading information (Table 4, model 2) increased the power of predicting disease-related mortality by 10% ($c = 0.82$; 95% CI = 0.76–0.88; $P < 0.0001$).

We conclude that the modified TNM staging system efficiently identifies four stages associated with proportionally increasing risk of death (Table 5).

Discussion

One of the most difficult questions to answer in clinical oncology is how to assess the prognosis of patients suffering from pancreatic endocrine tumors. Our study provides an applicable response originating from the use of our modified ENETS–TNM staging and Ki67 grading systems on a nonselected series of 274 patients affected by sporadic pancreatic endocrine tumors, who had been all subjected to surgical intervention (197 resections, 77 palliative or explorative), and therefore had the anatomical assessment of local or metastatic spread.

The results can be summarized as follows: (i) the original ENETS–TNM staging showed at multivariate analysis a significant difference between stage I/II and stage III or IV, as well as between stages III and IV, but did not find any difference in the probability of death between stages I and IIa and/or IIb tumors; (ii) the application of two modifications to the original TNM improved the prognostic ability of the system, assigning a proportional risk of death according to the stage at the time of diagnosis; (iii) a third modification consisting in the introduction of clinical–radiological parameters to assign T codes permits the application of this modified TNM system to all pancreatic endocrine tumor patients, as it circumvents the need of surgical exploration for staging purposes; (iv) the ENETS grading cut-offs of 2 and 20% only showed differences between G3 vs G1 and G2, but failed to demonstrate any difference

Table 4 Multivariate survival analysis on 274 patients affected by pancreatic endocrine tumor applying the modified TNM staging alone (model 1) or with the addition of modified grading with cut-off at 5 and 20% (model 2)

TNM Stage			Cumulative survival rate (Kaplan–Meier)			Relative risk			
Modified staging ^c	Original staging ^d	Number of dead patients (%)	1-year	5-year	10-year	Model 1 ^a		Model 2 ^b	
						HR (95% CI)	P-value	HR (95% CI)	P-value
I	I–IIa	2/108 (2)	100	100	93	1		1	
II	IIb	2/16 (12)	100	93	74	7 (0.99–49.73)	0.05	3 (0.26–32.43)	0.388
III	IIIa–IIIb	20/54 (37)	89	65	26	29 (6.75–124.21)	<0.0001	11 (2.29–52.35)	0.003
IV	IV	61/96 (63)	77	35	18	58 (14.14–237.82)	<0.0001	33 (7.46–148.2)	<0.0001
<i>Modified grading (Ki67 index)</i>									
G1 (0–5 %)		14/152 (9)	98	91	82	—		1	<0.0001
G2 (> 5 to 20%)		30/63 (48)	86	52	26	—		2 (1.11–4.56)	0.025
G3 (> 20%)		18/22 (82)	68	12	—	—		11 (4.58–24.47)	<0.0001
Concordance index ^e						0.72 (0.67–0.78)	<0.0001	0.82 (0.76–0.88)	

^aModel 1: Cox model adjusted for gender, age, site of neoplasm, type of neoplasm (functioning or not).^bModel 2: Cox model adjusted for all parameters used for model 1 plus Ki67% levels. Cox model for grading was adjusted for all parameters used for model 1 plus T, N and M variables (Table 1).^cModified staging as in Table 4 of this paper.^dENETS–TNM staging system.¹⁵^eHarrell *et al*, 1996.²⁰**Table 5** Modified clinical, radiological and pathological TNM staging of pancreatic endocrine tumors

Stage	T ^a	N ^b	M ^c
I	T1 or T2	N0–NX	M0
II	T3	N0–NX	M0
III	T4	N0–NX	M0
IV	Any T	N1	M1
	Any T	Any N	

^aT1, tumor limited to the pancreas and size <2 cm; T2, tumor limited to the pancreas and size between 2 and 4 cm; T3, tumor limited to the pancreas and size >4 cm; T4, tumor invasion of any adjacent structure (including duodenum, common bile duct and peripancreatic fat), documented by histology or clinical/imaging signs of malignancy (jaundice or duodenal bleeding due to infiltration, dilation of main pancreatic or common bile duct).^bNX, lymph nodes not assessed; N0, absence of lymph node metastasis; N1, invasion of regional lymph nodes.^cM0, absence of distant metastasis; M1, presence of distant metastasis.

in survival between G1 and G2 groups; (v) our modified tumor grading based on Ki67 immunohistochemistry with cut-offs at 5 and 20% permits prognostic stratification of patients within the same stage.

The validation of the ENETS proposed TNM system¹⁵ has been already addressed in four recent studies.^{16–19}

Ekeblad *et al*¹⁶ reported the analysis of 324 pancreatic endocrine tumors for which diagnosis according to WHO was available in only 241, whereas TNM data were available for 302 and grading for 93 patients. The study did not show any difference between stages I vs II or III, and only demonstrated a significant difference between stage IV (metastatic disease) vs any stage. Moreover, the

authors considered death for any cause as the main outcome for survival analysis. This resulted in a significant drop of survival after about 10 years for the WHO category of well-differentiated endocrine tumors in a disease for which only 70% of deaths are expected to be disease-related.¹⁹ Moreover, grading resulted significant only if poorly differentiated endocrine carcinomas were included.

Fischer *et al*¹⁷ presented a surgical series of 118 pancreatic endocrine tumors that did not show any difference between stages I and II, as well as between stages III (locally advanced disease) and IV (metastatic disease), possibly due to a short follow-up (median of 20 months). No grading was available in this series.

The study by La Rosa *et al*¹⁸ was limited to patients undergoing surgical resection with curative intent that comprised 155 patients. The authors used TNM and grading parameters modified with respect to those of ENETS¹⁵ and propose several models that combine histological and clinicopathological data in the attempt to stratify pancreatic endocrine tumor patients into groups with increasing malignant potential. The paper reaches no definitive or consistent conclusion on the best mixture of different histological and clinicopathological parameters to determine prognosis. Moreover, the parameters suggested in the modifications can only be obtained through analysis of surgical specimens and thus exclude a large number of patients.

Pape *et al*¹⁹ presented 202 foregut neoplasms, including 131 pancreatic endocrine tumors, 48 gastric and 23 duodenal neuroendocrine tumors. In this series, staging was based on imaging, as

pathological data were only available in 50% of cases that were unfortunately not identified. Grading was available in 78% of cases again not identified. The authors reported a significant difference between any stage *vs* stage IV, but did not demonstrate any difference between stages I *vs* II and stage II *vs* III. This is possibly due to the case mix used that prevented any possibility to discern the relative contribution of the diverse foregut endocrine tumors.

In summary, all four studies evidenced only clear-cut distinctions between higher and lower stages, but were of little help for intermediate-stage diseases, in part due to the weaknesses or selection bias highlighted above. Thus, they cannot provide concrete validation of the entire ENETS–TNM staging system.

Our study is based on the largest well-annotated series of pancreatic endocrine tumors available to date that deals with ENETS–TNM validation. The annotation for this series is particularly complete as it includes pTNM data obtained during surgery for all patients in the study, regardless of tumor resection.

Interestingly, when the ENETS–TNM was applied to our series, a significant difference was seen between stage I/II and the two higher stages, as well as between stages III and IV, something the other studies did not reveal. Moreover, multivariate analysis showed that tumor dimensions of over 4 cm and neoplastic invasion were independent prognostic factors in the absence of lymph node and distant metastasis.

On the basis of this latter finding, we introduced two modifications to the T code of the ENETS–TNM: (a) T3 was defined only by tumor dimensions; (b) T4 was defined by cancer invasion of any adjacent structure, including duodenum and bile duct. These modifications aim at providing a clear separation between potentially malignant diseases and cases for which malignancy is a certainty. Thus, T1–T3 categories describe the disease limited to the pancreas with increasing dimensions associated with a greater risk of malignancy, whereas T4 identifies the lesion as invariably malignant based on the detection of extrapancreatic structures infiltration.

A third modification was introduced with the aim of making TNM also applicable in cases where only clinical and imaging data are available. This was achieved by introducing clinical (jaundice or duodenal bleeding due to infiltration) or radiological signs of malignancy (dilation of main pancreatic duct or common bile duct). In fact, information such as jaundice is detected clinically, duodenal infiltration is detected by endoscopy and confirmed by biopsy, whereas tumor dimensions and the dilation of pancreatic and/or biliary duct are easily documented by imaging procedures.

The application of this modified ENETS–TNM provided the stratification of our 274 pancreatic

endocrine tumors (107 well-differentiated endocrine tumors, 139 well-differentiated endocrine carcinomas and 28 poorly differentiated endocrine carcinomas) into four different classes of risk corresponding to the four modified ENETS–TNM-based stages. These latter were identified as independent predictors of death at multivariate analysis. Patients with stages II, III or IV neoplasms showed a risk of death of 7, 29 or 58 times higher than patients having stage I neoplasms ($P < 0.0001$).

The next question is how to discern prognosis among carcinomas at the same stage. The measure of the growth pace of the neoplasia using grading scores based on the proportion of proliferating neoplastic cells is the clinically applicable answer. In fact, the application of such a grading system that uses Ki67 immunohistochemistry and cut-off values of 5 and 20%, suggested by the present study, efficiently discern prognosis among neoplasms at the same stage. Indeed, grading has already been shown to be useful for therapeutic decisions, particularly, in patients with advanced stage disease, as it has been recently reported.^{7,21}

The American Joint Committee on Cancer (AJCC) has recently proposed the application of the AJCC–TNM staging system devised for pancreatic ductal adenocarcinoma to pancreatic endocrine tumors.^{22,23} The difference between our modified ENETS–TNM and the AJCC–TNM consists in the definitions of T parameters, which impinges on the definitions of the stages (Table 6) that result in a strikingly diverse 5- and 10-year survival (Table 7). Our staging system already highlights differences in survival at 5 years between any two sequential stages. At the 5-year survival mark, the AJCC system only shows significant differences for stages III to IV, whereas differences between the other stages only become evident at 10 years, making it of a more limited clinical utility. At 5 years, AJCC-stage III survival shows a disappointing 4% difference from that associated with AJCC-stage II, implying the irrelevance of the involvement of large arteries in the evaluation of disease aggressiveness in pancreatic neuroendocrine carcinomas. Thus, AJCC-stage III and its underlying AJCC-T4 category serve no purpose. One quarter of AJCC-stage I patients are dead within 5 years, whereas our-stage I is identifying virtually all long-term survivors, among which only 7% are dead of disease at 10 years. The poorly discriminating performance of AJCC-stage I is conceivably due to the fact that it includes a large number of cases that would be classified in our-stage II. The AJCC approach, however, is perplexing from both a biological and a methodological point of view. The biological drawback resides in using the same system for pancreatic ductal adenocarcinoma, a fast proliferating and deadly disease, and pancreatic endocrine carcinoma that typically grows slowly and only impairs patient quality of life late in the course of the disease, even when metastatic. The methodological weakness resides in that the

Table 6 Differences in T code and stage definitions between modified ENETS and AJCC staging systems

	<i>Modified ENETS–TNM</i>	<i>AJCC–TNM</i>
<i>Code</i>		
T1	Limited to the pancreas, < 2 cm	Limited to the pancreas, ≤ 2 cm
T2	Limited to the pancreas, 2–4 cm	Limited to the pancreas, > 2 cm
T3	Limited to the pancreas, > 4 cm	Beyond the pancreas, no involved arteries ^a
T4	Beyond the pancreas ^b	Involvement of arteries (unresectable) ^a
<i>Stage</i>		
I	T1–T2 (limited to pancreas, ≤ 4 cm)	T1–T2 (limited to pancreas, any dimension)
II	T3 (limited to pancreas, > 4 cm)	T3 or N1 (outside of the pancreas)
III	T4 or N1 (outside of the pancreas)	T4 (involving large arteries, unresectable)
IV	M1	M1

^aCeliac axis or the superior mesenteric artery.^bInvolving any nonpancreatic anatomical structure (including duodenum, bile duct and fat).**Table 7** Differences in 5- and 10-year survival between modified ENETS and AJCC staging systems

<i>Survival</i>	<i>5-year (%)</i>		<i>10-year (%)</i>	
	<i>Modified ENETS–TNM</i>	<i>AJCC–TNM^a</i>	<i>Modified ENETS–TNM</i>	<i>AJCC–TNM^a</i>
Stage I	100	75	93	71
Stage II	93	64	74	46
Stage III	65	60	26	33
Stage IV	35	20	18	9

^aAJCC age-adjusted data from Table 24.3 of the AJCC cancer staging manual²² that is the same Table 2 of Bilimoria *et al.*²³ Percentages have been rounded to the nearest integer.

AJCC–TNM proposal is based on a single study that uses cancer registry data of 4793 cases collected from 1440 hospitals from which about one-third of the total cases and up to 50% of nonsurgical patients lacked T or N data and even assigned histology.²³

Although several papers and debates have been devoted to finding a response to which is the best available system to assess prognosis of well-differentiated neuroendocrine tumors, there is still a sort of confusion on the purpose and scope of the WHO classification and TNM staging systems. The main source of confusion comes from the fact that both systems use exactly the same variables: size, invasion, lymph node and liver metastasis. However, these parameters serve to reach a synthetic qualitative judgement in WHO that can thus assign a term to the disease, while they are independently evaluated and scored in the TNM system, which can thus quantitatively describe the extent of disease at the time of diagnosis. In short, WHO and TNM systems cannot be compared. They are different systems that serve different purposes. WHO is a classification system, and as such is intended to assign a name to a neoplastic process that describes its potential behavior, including benign, at-risk of malignant transformation or malignant; TNM is a cancer staging system, and as

such serves to assess prognosis of a malignancy that has already been given a name. To definitely overcome this confusion, the WHO classification of neuroendocrine tumors might be improved by considering any well-differentiated endocrine tumor as potentially malignant, because there is no parameter to assign bona fide ‘benignness’ to this disease. The WHO should also consider the exclusion of parameters such as venous, lymphatic or nerve invasion unless there is a clear consensus on their definition and significance. These parameters could better serve if included in the TNM staging system as occurs for any other cancer, thus serving to assess prognosis.

In conclusion, WHO classification of pancreatic endocrine tumors serves the primary purpose of assigning a diagnostic category with clinical significance. The TNM staging proposed by the ENETS, in whose creation the authors of this paper have participated, has shown validity in the distinction of prognostic classes among well-differentiated pancreatic endocrine tumors. The modifications proposed in this study ameliorate the clinical applicability and prediction of outcome of the ENETS–TNM staging. The grading system based on the proliferation index discerns prognosis among pancreatic endocrine tumors at the same stage when using the cut-offs suggested by this study.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

References

- Heitz PU, Komminoth P, Perren A, *et al*. Pancreatic endocrine tumours. In: DeLellis RA, Lloyd RV, Heitz PU, *et al*. (eds). World Health Organization Classification of Tumors. Pathology and Genetics of Tumors of Endocrine Organs. IARC Press: Lyon, 2004, pp 177–182.
- Hruban RH, Bishop Pitman M, Klimstra DSL. Endocrine Neoplasms. In: Silverberg SG, Sobin LH (eds). Tumors of the Pancreas. ARP AFIP: Washington, 2007, pp 251–304.
- Klöppel G, Rindi G, Anlauf M, *et al*. Site-specific biology and pathology of gastroenteropancreatic neuroendocrine tumors. *Virchows Arch* 2007;451(Suppl 1).
- Capelli P, Martignoni G, Pedica F, *et al*. Endocrine neoplasms of the pancreas: pathologic and genetic features. *Arch Pathol Lab Med* 2009;133:350–364.
- Hruban RH, Bishop Pitman M, Klimstra DSL. Endocrine Neoplasms. In: Silverberg SG, Sobin LH (eds). Tumors of the Pancreas. ARP, AFIP: Washington, 2007, pp 259–262.
- Oberg K, Eriksson B. Endocrine tumours of the pancreas. *Best Pract Res Clin Gastroenterol* 2005;19:753–781.
- Butturini G, Bettini R, Missiaglia E, *et al*. Predictive factors of efficacy of the somatostatin analogue octreotide as first line therapy for advanced pancreatic endocrine carcinoma. *Endocr Relat Cancer* 2006;13:1213–1221.
- de Herder WW, Niederle B, Scoazec JY, *et al*. Well-differentiated pancreatic tumor/carcinoma: insulinoma. *Neuroendocrinology* 2006;84:183–188.
- Falconi M, Plockinger U, Kwekkeboom DJ, *et al*. Well-differentiated pancreatic nonfunctioning tumors/carcinoma. *Neuroendocrinology* 2006;84:196–211.
- Jensen RT, Niederle B, Mitry E, *et al*. Gastrinoma (duodenal and pancreatic). *Neuroendocrinology* 2006;84:173–182.
- O'Toole D, Salazar R, Falconi M, *et al*. Rare functioning pancreatic endocrine tumors. *Neuroendocrinology* 2006;84:189–195.
- Capella C, Heitz PU, Hofler H, *et al*. Revised classification of neuroendocrine tumours of the lung, pancreas and gut. *Virchows Arch* 1995;425:547–560.
- Schindl M, Kaczirek K, Kaserer K, *et al*. Is the new classification of neuroendocrine pancreatic tumors of clinical help? *World J Surg* 2000;24:1312–1318.
- Schmitt AM, Anlauf M, Rousson V, *et al*. WHO 2004 criteria and CK19 are reliable prognostic markers in pancreatic endocrine tumors. *Am J Surg Pathol* 2007;31:1677–1682.
- Rindi G, Kloppel G, Alhman H, *et al*. TNM staging of foregut (neuro)endocrine tumors: a consensus proposal including a grading system. *Virchows Arch* 2006;449:395–401.
- Ekeblad S, Skogseid B, Dunder K, *et al*. Prognostic factors and survival in 324 patients with pancreatic endocrine tumor treated at a single institution. *Clin Cancer Res* 2008;14:7798–7803.
- Fischer L, Kleeff J, Esposito I, *et al*. Clinical outcome and long-term survival in 118 consecutive patients with neuroendocrine tumours of the pancreas. *Br J Surg* 2008;95:627–635.
- La Rosa S, Klersy C, Uccella S, *et al*. Improved histologic and clinicopathologic criteria for prognostic evaluation of pancreatic endocrine tumors. *Hum Pathol* 2009;40:30–40.
- Pape UF, Jann H, Muller-Nordhorn J, *et al*. Prognostic relevance of a novel TNM classification system for upper gastroenteropancreatic neuroendocrine tumors. *Cancer* 2008;113:256–265.
- Harrell Jr FE, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Stat Med* 1996;15:361–387.
- Aparicio T, Ducreux M, Baudin E, *et al*. Antitumour activity of somatostatin analogues in progressive metastatic neuroendocrine tumours. *Eur J Cancer* 2001;37:1014–1019.
- American Joint Committee on Cancer. Exocrine and endocrine pancreas. In: Edge S, Byrd D, Compton C, *et al* (eds). *AJCC Cancer Staging Manual* (ed Seventh). Berlin, Springer, 2010, pp 241–249.
- Bilimoria KY, Bentrem DJ, Merkow RP, *et al*. Application of the pancreatic adenocarcinoma staging system to pancreatic neuroendocrine tumors. *J Am Coll Surg* 2007;205:558–563.